Interaction between the CheY response regulator and the histidine-containing phosphotransfer (HPt) domain of the ArcB sensory kinase in *Escherichia coli*

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Abstract Bacteria have devised sophisticated His–Asp phosphorelay signaling systems for eliciting a variety of adaptive responses to their environment. The histidine-containing phosphotransfer (HPt) domain, found in many signal transduction protein, functions as a mediator of the His–Asp phosphorelay. The ArcB anaerobic sensor of *E. coli* contains such a HPt domain, although its function is not fully understood. In this study, we provide in vivo and in vitro evidence that the HPt domain is capable of interacting with the CheY receiver, which contains a phospho-accepting aspartate residue.

Key words: Phosphotransfer signaling in *E. coli*; ArcB anerobic sensor; HPt phosphotransfer domain; CheY response regulator

1. Introduction

Adaptive response systems in bacterial cells often involve two components of signal transduction proteins, sensory kinases and response regulators [1–4]. To date, instances of the two-component signaling systems have been described in a large number of bacterial species [5]. Furthermore, some have recently been discovered in eukaryotes, including plastids, protozoa, fungi, and plants [6]. Most of these signal transduction proteins contain one of the two common phosphotransfer signaling domains, transmitter and receiver [5]. The transmitters contain a crucial histidine residue, which is autophosphorylated in an ATP-dependent manner, whereas the receivers contain an aspartate residue which can acquire a phosphoryl group from the phospho-histidine of the cognate transmitter. Besides these domains, another common domain has recently been identified, which is also implicated in the phosphotransfer signaling [7–10]. This domain, referred to here as the histidine-containing phosphotransfer (HPt) domain contains a crucial histidine residue, which appears to function as a mediator of phophorelay by acquiring/transferring a phosphoryl group from/to other components (i.e., His–Asp phosphorelay) [11,12].

A typical HPt domain was first discovered in the *Escherichia coli* ArcB sensory kinase responsible for anaerobic regulation, which is unorthodox in the sense that it contains both a transmitter domain and a receiver domain in its primary amino acid sequence (see Fig. 1A) [7,8]. In ArcB, these two typical signaling domains are followed by a HPt domain. The *Bordetella pertussi* BvgS sensory kinase, involved in virulence regulation, is another example, which is structurally very similar to ArcB [9,13]. An even more striking instance of such a HPt domain has emerged from the yeast *Saccharomyces cerevisiae* osmoregulatory signaling system [10,14]. The Sln1p–Ypd1p–Ssk1p pathway represents yet another design of the phosphorelay, in which Ypd1p containing a HPt domain plays a crucial role in the His–Asp phosphorelay. Taking all these examples together, it was proposed that the HPt domains in a set of signal transducers function as a novel device for the signal transduction via multi-step phosphorelay [11]. However, exploration of the structure and function of the HPt domains is still at a very early stage.

Here we focused our attention on the structure and function of the HPt domain of ArcB (originally named ArcB^c; see Fig. 1) [7,8]. We recently determined the 3-dimensional structure of ArcB^c by X-ray analysis [15]. Based on this structural information, to address a relevant issue with regard to the structure and function of the HPt domain, we examined a possible interaction between the HPt domain of ArcB and the well-characterized response regulator, CheY, which is involved in the signal transduction responsible for the chemotaxis in *E. coli*.

2. Materials and methods

2.1. Bacteria and plasmids

E. coli K-12 strain DZ225 (F$^-$, Δ*envZ*, Δ*lacU169*, *araD139*, *rpsL*, *relA*, *thiA*, *flbB*) was constructed previously [16]. This strain carries an *ompC–lacZ* fusion gene on the chromosome. Strain CSH26 (Δ[*lac-pro*] *ara thi*) was also used, which is a wild-type with respect to the chemotactic behavior. Plasmids, pIA001 and pIA005, were constructed previously [7,8], both of which carry the *arcB* gene, but the latter has the mutations resulting in the His-292 to Leu, and Asp-576 to Gln substitutions.

2.2. Purification of ArcB^c and CheY

The ArcB^c consisting of the HPt domain was purified as described previously [7]. For the purification of CheY, a plasmid was constructed in this study, in which the CheY-coding region was placed under the T7-promoter on a versatile overexpression vector (pET-series, NOVAGEN, USA). From the cells carrying this plasmid, the CheY protein was purified by essentially the same procedures as those described for the purification of AtcB^c.

2.3. Enzyme assays

β-Galactosidase activity was determined by Miller's method with slight modifications, as described previously [7].

2.4. Phosphorylation experiment

Radioactively phosphorylated ArcB^c was prepared and purified as described previously [7,8]. Phosphotransfer experiment with phospho-ArcB^c and CheY was also carried out as described previously [7,8].

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2.5. Chemotactic assay on 'swarm' plates

For swarm assays, tryptone semisolid agar (1% tryptone, 0.5% NaCl, 0.3% agar) supplemented with 50 μg/ml ampicillin was used. An aliquot (3 μl) of fresh overnight culture in tryptone–glycerol medium (1% tryptone, 0.5% NaCl, 0.5% glycerol, and an appropriate cordon and energy source: 0.1 mM glucose or galactose) was spotted onto the semisolid agar plate, which was then incubated at 30°C for 20 h. For swarm assays under the micro-aerobic conditions, the swarm plate was incubated in GasPak Pouch™ (Becton Dickinson Microbiology, USA), at 30°C for 36 h.

3. Results and discussion

We examined a possible interaction between the HPt domain of ArcB and the CheY response regulator, in vivo and in vitro. The reason we wanted to address this particular issues is following (Fig. 1). The CheY response regulator interacts normally with the CheA chemotaxis sensor (Fig. 1B) [1]. Among a number of sensory kinases, the basic structural design of CheA is significantly different from that of other orthodox ones (compare with that of the ArcB transmitter domain in Fig. 1A) [5]. In fact, the short sequence surrounding the phosphorylated histidine site in CheA does not resemble those surrounding the phosphorylation histidine sites of other orthodox transmitters (Fig. 1C, compare with that of the ArcB transmitter domain containing His-1 site). Rather, we noticed that the CheA sequence in question is significantly similar to the sequence of His-2 site in the HPt domain (Fig. 1C). Second, recent NMR studies revealed that a domain of CheA containing the phosphorylated histidine site (named P1 domain) has a global fold of five α-helices that contains a four-helix bundle [17]. On the other hand, the recent X-ray analysis of the isolated HPt domain of ArcB revealed that this domain also consists of six α-helices containing a four-helix bundle with a kidney-like shape [15]. Interestingly, an inspection by superpositioning these two structures containing the active histidine sites of CheA and the HPt domain of ArcB, respectively, suggested that they are significantly similar to each other, based on this structural point of view too [15]. These facts led us to envisage the idea that HPt domain of

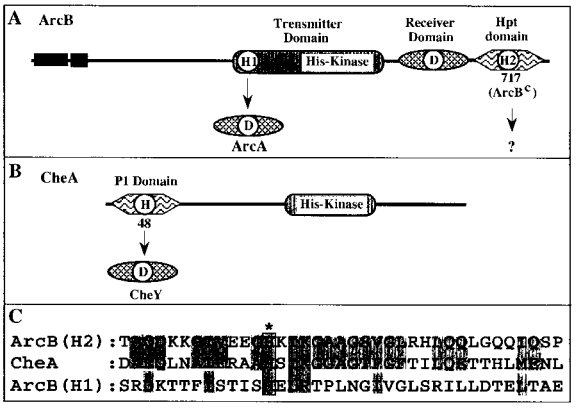

Fig. 1. Schematic representation of the structures of the ArcB and CheA sensory kinases. The ArcB sensor contains three signaling domains, one of which is the C-terminal HPt domain (A). The CheA sensor has the N-terminal phospho-donor histidine site (called P1 domain), in addition to the histidine-kinase domain (B). In these structures, the active histidine/aspartate sites are indicated schematically. These active histidine sites were compared in terms of their amino acid sequences. Similar or identical amino acids among them were shaded.

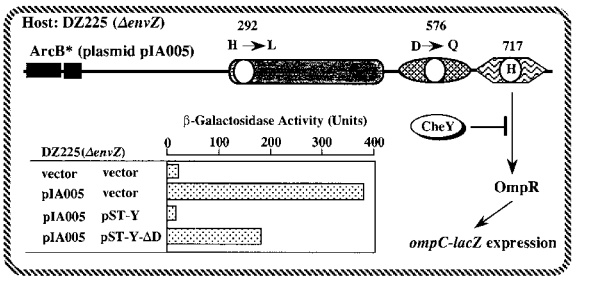

Fig. 2. An experimental design to examine a possible in vivo interaction between CheY and the HPt domain of ArcB. The inset data shows β-galactosidase activities, determined for strain DZ225 carrying each combination of plasmids, as indicated. Details were given in the text.

ArcB might interact with CheY, as in the case of CheA. To gain a new insight into the structure and function of the HPt domain, in this study we tested this idea by taking both in vivo and in vitro approaches.

Based on the following experimental rationale, an interaction of CheY and the HPt domain was first assessed in vivo. We previously demonstrated that the HPt domain of ArcB is capable of functioning as a phosphodonor in vivo. Here we used the previously constructed plasmid (named pIA005), which specifies a mutant ArcB protein (named ArcB*) containing His-717, but lacking both His-292 (His to Leu substitution) and Asp-576 (Asp to Gln substitution) (Fig. 2). In other words, the mutant ArcB* protein has the functional HPt domain, but its transmitter and receiver domains are presumably inactive. Provided that ArcB* is overexpressed through the multicopy plasmid, it is capable of phosphorylating the OmpR response regulator, even in the absence of its cognate EnvZ sensory kinase [7,8]. Consequently, the resultant phospho-OmpR activate an *ompC–lacZ* fusion gene in strain DZ225 (*ΔenvZ*), as schematically shown in Fig. 2 (note that the *ompC* gene is one of the targets of the OmpR transcriptional activator). This particular phenomenon was used as a hallmark of the in vivo function of the HPt domain of ArcB, as follows. Strain DZ225 carrying an *ompC–lacZ* fusion gene exhibits the Lac$^-$ phenotype due to an *envZ* deletion (see the inset graph in Fig. 2). When the strain was transformed with pIA005 carrying the *arcB** gene, the β-galactosidase activity of the transformant increased substantially, as explained above. However, when a multicopy plasmid (pST-Y) carrying the *cheY* gene was further introduced into the transformant, the level of β-galactosidase dramatically decreased to a basal level. When a mutant *cheY* gene, encoding a protein with the Asp-13 to Asn substitution, was introduced (pST-Y-ΔD), this effect was largely abolished. This in vivo result was best interpreted by assuming that the CheY receiver is capable of competing with the OmpR receiver by titrating the phosphoryl group from the HPt domain of ArcB. This suggest that CheY may interact with the HPt domain in vivo, as schematically shown in Fig. 2.

An in vivo interaction of CheY and the HPt domain was further assessed vis á vis (Fig. 3). Strain CSH26 is a wild-type with respect to the chemotaxis. This strain was evaluated in terms of its chemotactic behavior on soft agar 'swarm' plates containing glucose or galactose as carbon sources, showing that the cells formed large rings indicative of the chemotactic behavior. However, when CSH26 was transformed with either pIA001 carrying the wild-type *arcB* gene or pIA005 carrying

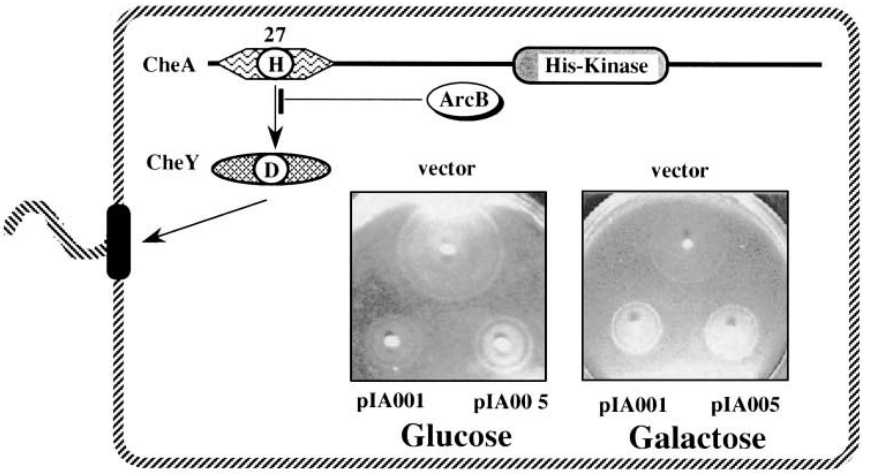

Fig. 3. An experimental design to examine a possible in vivo interaction between CheY and the HPt domain of ArcB. The inset shows the results of the chemotactic swarm test, which was conducted for strain CSH26 carrying each plasmid, as indicated. Details were given in the text.

the mutant *arcB** gene, the size (i.e., diameter) of the chemotactic ring was remarkably reduced (Fig. 3, see inset). An even more striking effect on the chemotaxis was observed, when we carried out the similar experiments under a micro-aerobic condition for growth, under which conditions the ArcB anaerobic sensor was expected to be more activated (data not shown). These observations were compatible with the idea that the overexpression of ArcB (particularly of the HPt domain) jams the CheA–CheY phosphotransfer signaling that is essential for the chemotactic behavior, as schematically shown in Fig. 3. The overexpression of ArcB may result in a hyperphosphorylation of CheY, thereby interfering with the normal chemotaxis. Alternatively, the normal interaction between CheA and CheY may be disturbed by a competitive interaction between ArcB and CheY. In any case, this in vivo result is also indicative of the interaction of CheY and the HPt domain of ArcB.

To directly evaluate the above in vivo results, the CheY protein and the HPt domain of ArcB (named ArcB^c) were both purified to near homogeneity (Fig. 4A). Radioactively phosphorylated ArcB^c (presumably at His-717) was then prepared, as described previously (Fig. 4B, lane 1) [7,8]. This purified phospho-ArcB^c was incubated for 5 min at 20°C in a buffer containing 50 mM KCl and 5 mM $MgCl_2$ (lane 2), and then CheY was added. At very short intervals, the samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by autoradiography (Fig. 4B). These data were also shown in a quantitative manner (Fig. 4C). As shown in Fig. 4B,C, phospho-ArcB^c disappeared within a minute, and CheY was phosphorylated transiently. Based on the current knowledge with regard to the two-component phosphotransfer signaling mechanism [1], this was interpreted as meaning that the phosphoryl group on ArcB^c was transferred rapidly onto an aspartate residue of CheY (presumably at Asp-13), then the phosphoryl group was released rapidly from CheY. We previously demonstrated that the phosphoryl group on ArcB^c can be transferred onto the purified OmpR protein under essentially the same in vitro conditions as used here [8]. In this respect, it is worth mentioning that the phosphoryl group on ArcB^c appears to be transferred much more rapidly onto CheY than onto OmpR (>10 times faster). In any event, this in vitro result demonstrated that the HPt domain of ArcB is capable of interacting with CheY in such a way that the efficient phosphotransfer reaction occurs between them.

The HPt domain is a newly emerged phosphotransfer device, which is postulated to play a crucial role in the His–Asp phosphorelay [11]. The in vivo and in vitro results presented

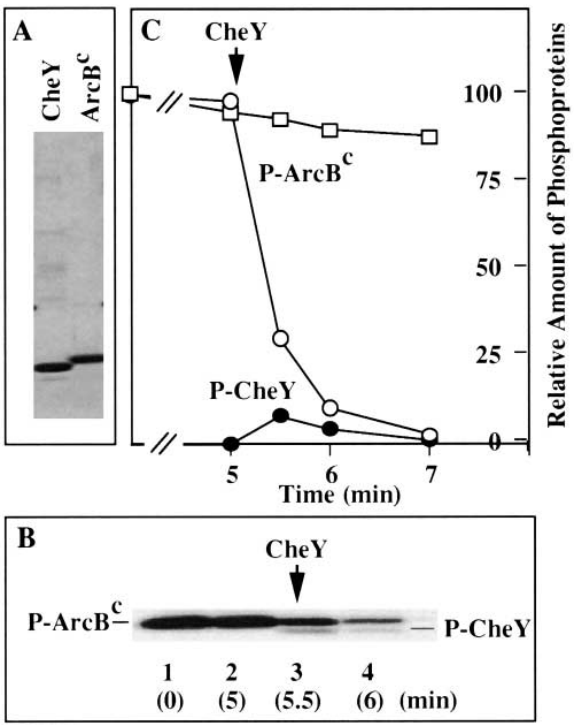

Fig. 4. In vitro phosphotransfer experiment. A: An electrophoretogram of the purified CheY and ArcB^c. B: Radioactivity phosphorylated ArcB^c was purified (lane 1). This was incubated for 5 min (lane 2), and then added the purified CheY at the time indicated. At short intervals, samples were analyzed by SDS-PAGE, followed by autoradiography (lanes 3 and 4). C: Data of B were quantitatively represented. Phospho-ArcBc was incubated in the presence of CheY, and the amounts of phospho-ArcBc (○) and phospho-CheY (●) were measured quantitatively. As a control, phospho-ArcBc (□) was also incubated in the absence of CheY, which is not shown in (B).

in this study provided evidence that the HPt domain of ArcB is capable of interacting with CheY in such a way that an efficient phosphotransfer occurs between them. The 3-dimensional structure of CheY has been well characterized, in which it folds into $(\alpha/\beta)_5$ globular fold [18]. A preliminary model building analysis showed that the molecular structure of the HPt domain of ArcB is well complementary to the molecular surfaces of CheY (data not shown). The concavity of the kidney-shaped molecule of the HPt domain fits well against the round surface of the globular CheY molecule. This structural view is well consistent with the results here, but verification must await further experimentation. In any case, our results support the general view that the HPt domains, found in many signal transduction proteins, may be capable of interacting with the receiver domains via the His–Asp phosphorelay. In this respect, the classical phospho-donor histidine site of CheA can be envisaged as an instance of the HPt domain.

The physiological relevance of the observed interaction between the ArcB and CheY signal transducers is not yet clear. We examined an *arcB* deletion mutant (a derivative of CSH26) in terms of its chemotactic behavior under both the aerobic and micro-aerobic conditions. Under the aerobic conditions, this mutant showed the chemotactic behavior on swarm plates as normally as in the case of the wild-type. Under the micro-aerobic conditions tested, however, the chemotactic ring formed by the mutant was reproducibly small, as compared with that formed by the wild-type (data not shown). This preliminary observation must be evaluated cautiously, because the defect in the anaerobic sensor should result in changes in several physiological states, including the growth rate. Nevertheless, it is tempting to speculate that the phosphorelay signal through the HPt domain of ArcB may be linked to the chemotactic signal under as yet unidentified conditions.

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References

[1] J.S. Parkinson, Cell 73 (1993) 857–871.
[2] Hock, J.A. and Silhavy, T.J. (1995) in J.A. Hoch and T.J. Silhavy (Eds), Two-component signal transduction, American Society for Microbiology, Washington, DC.
[3] Stock, J.B., Surette, M.G., Levit, M. and Stock, A.M. (1995) in J.A. Hoch and T.J. Silhavy (Eds), Two-component signal transduction. American Society for Microbiology, Washington, DC, pp. 25-51.
[4] J.B. Stock, A.D. Ninfa, A.M. Stock, Microbiol. Rev. 53 (1989) 450–490.
[5] J.S. Parkinson, E.C. Kofoid, Annu. Rev. Genet. 26 (1992) 71–112.
[6] L.A. Alex, M.I. Simon, Trends Genet. 10 (1994) 133–139.
[7] K. Ishige, S. Nagosawa, S. Tokishita, T. Mizuno, EMBO J. 13 (1994) 5195–5202.
[8] M. Tsuzuki, K. Ishige, T. Mizuno, Mol. Microbiol. 18 (1995) 953–962.
[9] M.A. Uhl, J.F. Miller, EMBO J. 15 (1996) 1028–1036.
[10] F. Posas, S.M. Wurgler-Murphy, T. Maeda, E.A. Witten, T.C. Thai, H. Saito, Cell 86 (1996) 865–875.
[11] J.L. Appleby, J.S. Parkinson, R.B. Bourret, Cell 86 (1996) 845–848.
[12] M. Inouye, Cell 85 (1996) 13–14.
[13] B. Arico, J.F. Miller, C. Roy, S. Stibits, D. Monack, S. Falkow, R. Gross, R. Rappuoli, Proc. Natl. Acad. Sci. USA 86 (1989) 6671–6675.
[14] T. Maeda, M. Takekawa, H. Saito, Science 269 (1995) 554–558.
[15] M. Kato, T. Mizuno, T. Shimizu, T. Hakoshima, Cell 88 (1997) 717–723.
[16] S. Nagasawa, K. Ishige, T. Mizuno, Mol. Microbiol. 6 (1992) 799–806.
[17] H. Zhou, D.F. Lowry, R.N. Swanson, M.I. Simon, F.W. Dahlquist, Biochemistry 34 (1995) 1358–1370.
[18] A.M. Stock, J.M. Mottonen, J.B. Stock, C.E. Schutt, Nature 337 (1989) 745–749.